

Atty Dkt. No.: RIGL-011
USSN: 09/710,058

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph starting on line 6 of page 73 as follows:

For preparation of whole-cell lysates, cells were counted, collected, washed in PBS, and lysed by freeze-thaw/vortexing in lysis buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100) with added complete protease inhibitor cocktail (Boehringer Mannheim, Chicago, Ill.). Lysate cleared by centrifugation was resolved on 4-12% NuPage SDS polyacrylamide gels (Novex, San Diego, Calif.) as per the manufacturer's recommendations. For immunoprecipitations, antibody conjugated agarose beads were added to the cell lysate, incubated for 4 h. The beads were washed in lysis buffer, and samples separated by SDS PAGE as above. Samples transferred to PVDF membranes were blocked overnight at 4.degree. C. using PBS buffer containing 10% Milk, 0.1% Tween20. Primary antibodies (polyclonal flag-probe, Santa Cruz Biotechnology, Santa Cruz, Calif.) were used at a 1:2000 dilution while secondary antibodies were used at a 1:5000 dilution. Membranes were developed using ECL plus enhanced chemiluminescence kit (Amersham Pharmacia, Piscataway, N.J.) and Hyperfilm ECL film (Amersham Life Sciences, Buckinghamshire, UK). For comparative Western blot analysis, GFPs containing a C-terminal flag were used. Exposed film was scanned with a Hewlett Packard (Palo Alto, Calif.) ScanJet 4C scanner and band intensities were integrated using the program NIH Image (see <http://rsb.info.nih.gov/ni-image/about.html>).

Please amend the paragraph starting on line 30 of page 73 as follows:

CD spectra were recorded as described in Gururaja *et al.*, Chem. Biol. (2000). Spectra were recorded between 200 and 250 nm at 0.2 nm intervals with a time constant of 1s. Data was collected from five separate scans and averaged. The protein concentrations were in the range of 5 to 10 μ M, as determined by the Lowry method, described in Lowry *et al.*, J. Biol. Chem. (1951) 193: 265-275. Protein solutions were made in 10mM phosphate buffer containing 100mM KF at pH 7.5, and were diluted in the same buffer to yield appropriate the final concentration. The thermal denaturation was measured at 218 nm over a range of 4-98°C with a temperature

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step of 2° C, a 2 minute equilibration time, and a 60 s signal averaging time. The apparent T_m was also determined by fitting the data to a logistic sigmoid equation using the Levenberg-Marquardt algorithm in Ultrafit (Biosoft, Cambridge, UK). In addition, the apparent T_m was determined as the maximum of the first derivative of the CD signal with respect to temperature. Both methods of T_m calculation agreed well. CD spectra were deconvoluted with the program CDNN (CD neural network) downloaded from ~~<http://bioinformatik.biochemtech.uni-halle.de/cdnn/index.html>~~.